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Some Physical-Chemical Properties of Lipoids

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SOME PHYSICAL-CHEMICAL PROPERTIES OF LIPOIDS

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GERTRUDE NIEDERMAN

B. S. University of Illinois, 1908

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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C. G. MacArthur
In Charge of Major Work

W. A. Noyes
Head of Department

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I. INTRODUCTION

Very little is known at the present time of the chemical changes that lipoids undergo during the cell metabolism. Their importance from a chemical point of view, however, can not be doubted when one considers that some of them contain all the elements that make up the living protoplasm, their general resemblance to the proteins, which were till now considered the life-giving substances, and their lability, which makes them very suitable material to supply the constant demand in the cell due to the breakdown of tissues. But it is due to their physical properties that the lipoids are considered so important, because their relation to the life processes has been more evident, although the exact nature of their functions are still unknown.

Lipoids are substances that resemble the fats very closely in their physical properties, especially in their solubilities in organic solvents. According to their chemical composition they can be divided into four groups:

I. Cholesterol Group, containing C, H, O

II. Cerebroside Group, containing C, H, O, N

III. Phosphatid Group, containing C, H, O, N, P

IV. Sulphatid Group, containing C, H, O, N, P, S

Other substances are sometimes classed under lipoids. In fact, Overton included all substances that could be extracted from the tissues by means of ether and similar organic solvents under that head. Bang[1] includes all neutral fats in his classification. The fourth group given above has so

far not been included in any classification of the lipoids. Their occurrence wherever protagon is found, with the cerebrosides and sphingomyelin, their means of extraction and their general properties, give them a place among the lipoid bodies. It is evident that Overton's and Bang's system of classification would make the term "lipoid" indefinite and therefore of no special meaning in biology. The term has recently been limited to only those substances that have the physical properties of fats and are usually of colloidal nature. The above classification has been made with that limited sense of the term in mind. The properties of those members of each group with which this paper is concerned will be briefly outlined below before we go on with the consideration of the theories of lipoid function in the living organism.

Cholesterol Group - C, H, O

Cholesterol. This substance is obtained by crystallizing out of hot alcohol as white characteristic plates. It is insoluble in water, soluble in hot alcohol, less soluble in cold alcohol, soluble in acetone and less soluble in petroleum ether. It is very solluble in ether. It is easily soluble in benzene and chloroform. Cholesterol is a very stable body and is found as a constant constituent of all cells. It is also found in nearly all body fluids. Its exact structure is as yet not definitely known. Koch[2] gives the following formula:

$$(CH_3)_2C_{20}H_{31} \qquad CH$$

$$CH_2 \quad CH_2 \quad CH_2$$

$$CHOH$$

being of a terpene structure. It is a monovalent, simple, unsaturated, secondary alcohol, whose hydroxyl group is between two methyl groups.

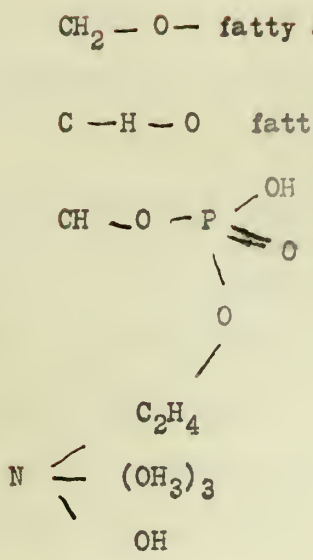
The Cerebrosides - C, H, O, N

Cerebron. This substance belongs to the group of cerebrosides and contains C, H, O, N, in its molecule but no phosphorus or sulphur. It was given the empirical formula $C_{44}H_{79}NO_8$ by Thierfelder,[3] and is probably the same substance that Thudicum[4] calls Phrensosin and Koch[5] calls Cerebrin. The difference in these substances is probably due to slight impurities, as the preparation of pure cerebron is a very difficult one. When hydrolized with sulphuric acid (10%), cerebron yields the methyl ester of cerebronic acid, $C_{25}H_{50}O_8$, another substance which contains nitrogen, sphingosine, and a sugar, galactose. Cerebron is obtained as a white powder by crystallizing out of hot alcohol. It is a very stable substance and is not decomposed by light or warm solvents. It is insoluble in water and in most cold solvents, but is soluble in cold pyridine. It is soluble in hot alcohol and chloroform. It is optically active in the last solvent, being dextrorotatory.

The Phosphatids - C, H, O, N, P

Thudicum classified five groups of phosphatids on the basis of a phosphorous and nitrogen ratio. As we are here concerned only with the most important phosphatids, namely, those that are found in greatest quantity in the tissues and especially in the nervous tissues, his classification will not be discussed at length. The phosphatids under consideration are lecithin, kephaline, and sphingomyelin. The first two belong to Thudicum's[6] first group, containing phosphorus and nitrogen in the ratio of 1:1. The third substance contains nitrogen and phosphorus in the ratio of 2:1, and belongs to Thudicum's second group of phosphatids.

Lecithin. The structure of this substance is as yet not definitely known. It is usually represented by the formula given below.



Whether all the nitrogen is contained in choline as the above formula indicates is doubtful, however. One of the fatty acids in its molecule is unsaturated, which gives lecithin very unstable properties, being very easily oxidized and hydrolized. Lecithin is obtained as a yellowish white to an orange brown waxy substance on precipitation with acetone from an ether solution. It is very soluble in ether, alcohol, benzene, chloroform, petroleum ether, and toluene. It is insoluble in acetone and in ethyl acetate. It takes up water very readily, forming a swollen mass which on further addition of water forms an opaque, colloidal solution.

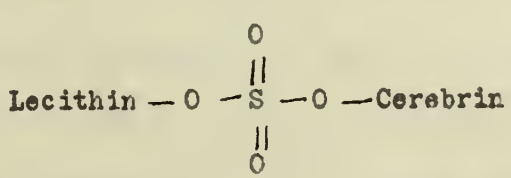
Kephaline. The physical properties of this phosphatid are very similar to those of lecithin. The chemical structure of kephaline is as yet unknown, but it seems to be similar to that of lecithin, the main difference being in the nature of the nitrogenous base, and in its unsaturated fatty acids, the latter being more unsaturated in kephaline. Kephaline is therefore even more unstable a substance than lecithin. As obtained by precipitating with acetone or alcohol, it is a yellowish white powder when dry. When exposed to the light and air it readily becomes oxidized and

turns a deeper yellow. Its behavior to water and organic solvents is the same as that of lecithin with the exception of alcohol, in which kephaline is insoluble.

Sphingomyelin. The structure of this substance is unknown, but it is known that it does not contain any unsaturated fatty acids. It is therefore very stable. It is soluble in hot alcohol, but crystallizes out on cooling in white rounded masses. It is soluble in chloroform and a mixture of chloroform and methylalcohol. It is soluble in warm pyridine but crystallizes out on cooling. It is insoluble in ether and in acetone.

The Sulphatids - C, O, H, N, P, S

Very little is known about the sulphatids. Most investigators have entirely lost sight of it. Probably because of the very inefficient methods used in separating the lipoids from each other, some of the substances were lost in the mother liquor or were not extracted from the brain. Thus Noll, Cramer, Kossel, and Koch find sulphur in protagon, while other investigators have not found any sulphur in their preparation. Thudicum was the first to speak of a sulphur-containing substance found in the brain and called it cerebrosulphatid, probably because he did not separate it from the cerebrosides very completely. He gives no definite information, however, about his sulphatide. According to Koch,[7] sulphatid has the formula given below.



The sulphuric acid, according to him, is joined to cerebrin in ester combination, for whenever sulphuric acid is split off, sugar is also found. According to Levene[8], sulphatid does not contain any phosphorus. Levene remarks that the properties of sulphatids are very much like those of the other lipoids, but he does not discuss these properties. The sulphatids are insoluble in most cold solvents that are used to dissolve other lipoids. They are soluble in warm pyridine and a warm mixture of methyl alcohol and chloroform. It is a yellowish white powder when obtained by crystallizing from pyridine.

A review of the properties of the substances considered above will make it evident that their classification under the one main group "Lipoids" can not be on a chemical basis. As stated above, it is their physical properties that make these substances so closely allied and so important to the biological processes. From our present knowledge of the lipoids, their chemical importance is as yet largely in the dark. Why are these physical properties so important? The answer to this question lies in the fact that the osmotic properties of the living cell are closely connected with the physical properties of the lipoids.

If a copper sulphate solution and pure water are carefully stratified over each other and allowed to stand, it will be found that the copper sulphate solution slowly diffuses into the pure water, and the latter diffuses at a somewhat higher rate into the copper sulphate solution. This process continues until only one homogeneous solution is obtained. When, however, a membrane which is permeable to the pure solvent but impermeable to the salt in solution, in this case copper sulphate, is placed between the two liquids, water will be found to go from the pure water side thru

the membrane and into the copper sulphate until the pressures on both sides of the membrane are equal. A membrane which is permeable to the solvent but not to the solute is called a semi-permeable membrane. The nature of semi-permeability will be taken up more fully later. The attraction (we will call it for the present) that the copper sulphate has for its solvent, water, is called the osmotic pressure. This pressure can be measured by a mercury manometer attached to the cell described above. The cause of osmotic pressure is explained in various ways. Vant Hoff[9] and his followers think that it is due to the bombardment of the molecules in solution against the membrane. Traube[10] and others think that osmotic pressure is a hydrostatic pressure due to the entrance of the solvent into the cell. The cause of the entrance of the solvent into the cell they explain differently - chemical affinity, capillary attraction, or, as Traube thinks, difference in surface tension between the two liquids, being the cause. All agree, however, that the semi-permeability of the membrane is essential, the value of the osmotic pressure being directly related to the difference in permeability of the membrane to the substances on the opposite side of the membrane.

A plant cell consists of a protoplasmic membrane surrounding the inner sap and a cellulose membrane surrounding the protoplasmic membrane. The latter is found to be semi-permeable and when we place a living cell in a liquid medium we have the same condition we had in the above example with the artificial membrane. The plant cell is impermeable to the salts it holds within its protoplasmic membrane, but permeable to the water within and without the cell. Water will then go in or out of the cell depending on the difference in attraction between the salts inside and the salts outside of the cell. It is evident then that osmosis is a great factor in

biological processes, as the transference of material from an outside medium into the cell and vice versa is a direct result of it.

II. A CRITICAL REVIEW OF PREVIOUS WORK

The function of a semi-permeable membrane was recognized as far back as the eighteenth century. In the nineteenth century Dutrochet and Vieroldt for the first time studied quantitatively the osmotic pressures produced by different salts, using different kinds of membranes. The membranes they used were not semi-permeable, but the results were important, as they brought out the fact that the nature of the membrane and the concentration of the salts used were factors of the osmotic pressure produced. The next great step in the study of osmotic pressures was taken by Moritz Traube,[11] who was able to make artificial semi-permeable membranes. Graham discovered that membranes could be used in separating substances from each other. He used animal membranes and called those substances that could pass thru such membranes, crystalloids, and those substances that could not pass thru, colloids. This discovery gave to those interested in this field of work the important fact that colloids could not pass thru a colloidal membrane. Traube's precipitation membranes were a direct result of this discovery. He formed these membranes by allowing two substances to precipitate each other. The result was a colloidal semi-permeable membrane, since the precipitate formed was not permeable to either of the colloids forming the precipitate. Traube's membranes were not very practical, however, for they could not withstand an

appreciable pressure. Pfeffer[12] greatly improved Traube's method of precipitating these membranes, by allowing the precipitation to occur within the pores of earthenware cups, which gave support to the membranes formed. These membranes could stand pressures of several atmospheres and so were of much more practical value than were Traube's membranes. In this modification of Traube's cell, Pfeffer imitated the plant cell, the latter having a cellulose wall to support the protoplasmic membrane. The Pfeffer cell has greatly been improved upon recently by Berkley, Hartley, Morse,[13] and Frazer, so that osmotic pressure measurements can now be accomplished with great accuracy. These improvements consist chiefly in details, the principle being the same except that their membranes are electrically deposited.

Besides the osmotic pressure experiments that were carried on with artificial membranes, many of which were made in the interests of physiology (by Dutrochet, Traube, Pfeffer), experiments were made with living cells. These were in the nature of plasmalytic experiments, in which the degree of plasmolysis of the cell was the measure of the osmotic pressure produced. The essentials of such an experiment are as follows: If a cell is placed in a solution of higher concentration than the concentration of the salts within the cell, water will pass thru the semi-permeable wall but no salt from without will enter. The cell loses, therefore, in volume and the protoplasm shrinks from the cell wall. If the cell is placed in a solution of lower concentration than the concentration of the salts within the cell, water will pass from the solution into the cell. The cell will gain in volume, and if the walls are not strong enough to stand the pressure, they will burst. DeVries[14] used this method very extensively. The same method has also been used by Hamburger[15] on animal cells. Hamburger used the red

corpuscles. If defibrinated blood is placed in a solution of higher concentration than the salts within it, the blood corpuscles will settle to the bottom and the liquid above will be colorless. If the concentration of the solution outside is lower than the concentration of the corpuscle contents, the corpuscles will not settle to the bottom. A concentration can be obtained then for all salts that do not act harmfully on the corpuscles at which the latter will just settle. The osmotic pressure for isotonic solutions found by this method agreed closely with that found by DeVries by using vegetable cells.

The experiments of DeVries and Hamburger show clearly that the living cell behaves similarly to a "Pfeffer" cell, and that absorption of food stuffs from the intestinal canal, its transference from the blood to the lymph and to the tissues, and also all gland secretions, can be explained on an osmotic phenomena basis. The speculations as to the transference of materials in the organism have been of various nature even in olden times, but so little was known of the anatomy of the human body or of lower animals that notions of the process were very false ones indeed, and it was not until blood and lymph vessels were found to be part of the anatomy that somewhat rational ideas of adsorption developed. We find even as late as the eighteenth century the belief that the chyle vessels communicated directly with the intestinal canal thru some openings. Another prevalent view of the time was that the intestinal walls imbibed the food material and that its transference to the blood and from there to the lymph and to the tissue fluids, took place thru a process of filtration. The evidence for this view was the fact that blood and lymph contain the same amounts of certain substances. The evidence in favor of the vitalists'

point of view, however, is evidence against the filtration theory. The vitalist theory was propounded by Bichat and Borden. It held that the intestinal walls brought about absorption thru some vital force within its cells which had no connection with the physical chemical properties of the cell. There are many physiological facts which seemed to uphold the view of the vitalists. It is a well-known fact the blood corpuscles contain more phosphorus and potassium than does the blood plasm, while the latter is richer in sodium salts. Also, the urine contains more urea than does the blood from which it is transferred. We have in both these cases examples of substances going apparently from a place of lower to a place of higher concentration, which is contrary to the laws of osmosis, according to which the flow is from lower to higher concentration. The fact that salts can pass from the intestine into the blood but not in the opposite direction was also in favor of the vitalists' theory, as that phenomenon could not be explained on a basis of physical permeability of the cell wall. According to their view, each organ was the seat of a specific vital force. This view is still held by many. Thus Höber[16] distinguishes between physical permeability of the cell wall and physiological permeability. These apparently mysterious phenomena have, however, been explained in a very natural way recently. Moore and Roaf[17] explain these facts on a basis of affinity between the protoplasm and the substances entering the cell. According to this view, when a substance which enters the cell can go into some combination with a part of the protoplasmic complexes, some more of the substance will enter to establish equilibrium. This process will continue until no more of the substance combines with the protoplasm, and then only such amount will enter as will be in equilibrium with the substance on the outside of the cell. The substance entering the cell may exist there,

then, in two forms, one combined with parts of the protoplasm and the other uncombined. The total concentration of the substance in that case can be higher in the cell than without, where it had its origin. Such a view is not contrary to a purely physical permeability and an osmotic explanation.

We now come back to the osmotic experiments on cells which were beginning to make clear the nature of resorption in such a wonderful manner. In his plasmalytic experiments DeVries found that not all substances would cause plasmalysis. To explain this difference in plasmalytic properties of salts and to explain such apparent contradictions of cell osmosis as the higher concentration of a substance in a secretion than in the gland where it had its origin, Overton[18] undertook a series of experiments to study the osmotic properties of living cells, the results of which have been of more consequence to biology than any other series of experiments along this line.

Overton used two methods in his experiments on living cells. The first was the plasmalytic method, for which he used roots of Hydrocharis, which he dipped into different solutions of varying concentrations. He also used this method on animal cells, using the tadpole and blood corpuscles as the objects of experimentations. In his experiments on alkaloids, he made use of the well known fact that tannic acid, which is found in all plant cells, precipitates alkaloids. He could then study the rate of entrance of the alkaloid into the cell by the rapidity of precipitation. The substances he used were of widely different nature, inorganic and organic salts, acids, and bases, narcotics, hypnotics, and dyestuffs. The results of these experiments threw great light on the physical-chemical nature of the process of adsorption and secretion. They showed that all substances that could enter the cell were soluble in fats, oils, and their

solvents, and that the rate of entrance was in direct relation to the degree of solubility of the substance in fats, oils, ether, and similar substances; and that those substances which were more soluble in water than in fats and ether could not enter the cell. Electrolytes did not enter the cell, while of the organic substances that entered, certain groups influenced the molecule very greatly in their rapidity of entrance. The order of magnitude of this influence is given below.

1. Amino acids
2. Carboxyl Group
3. Acid Omid Group
4. The Alcoholic Hydroxyl Group
5. The Aldehyder Group

The chemical constitution of the substance in so far as it is related to the solubility of the substance in fats and ether is also related to the entrance of the substance into the cell.

From these results it was very natural for Overton to postulate his "Lipoid Theory." Overton's process of reasoning which led to this theory were as follows:

1. Only those substances enter the cell that are soluble in fats, oils, and fat solvents.

2. The cells behave osmotically very similarly to a Pfeffer cell with a precipitation membrane.

3. From the two foregoing facts, the cell must be surrounded by a membrane of fat like nature.

4. All cells contain cholesterol and lecithin, substances that are very similar to the fats in their solubility properties, and in which the substances that are soluble in fats are found to be soluble.

5. The cell, therefore, must have a cholesterol, lecithin (lipoid) membrane around it, which thru its selective solubility properties gives the cell its osmotic properties.

To sum up these points, the Lipoid Theory, as stated by Overton, says that only those substances that are soluble in a lipoid membrane can enter a living cell.

A review of the substances that Overton found could enter the cell will bring out the interesting point that most substances that serve as food for the organism can not enter the cell, while those substances that are in a varying degree harmful to the cell processes, as hypnotics and narcotics, enter the cell very rapidly. It would seem, then, in the light of the above facts, that the cell, contrary to all other things in nature, was not constructed according to a very wise plan. But the cells, in process of growth, reproduction and maintenance, must get food, and as a matter of fact analyses of cells show the presence of nutritive materials within the cell. How does this food get into the cell if it must get thru a membrane that is impermeable to it? To meet this objection this theory was later modified somewhat, by giving the cell membrane a mosaic-like structure, parts of which were lipoid substances and parts other substances, as proteins, thru which the lipoid-non-soluble food could pass. Other investigators are now of that opinion. In fact, analysis of the stromata of the red corpuscles show the presence of proteins as well as lipoids. The mechanism of absorption of foods to which the lipoid membrane is impermeable has been explained by Starling[19] as due to the combination of the substance trying to gain entrance with parts of the membrane, thus forming a lipoid soluble substance. According to this view, whether a substance will enter the cell or not will

depend on the equilibrium of the cell protoplasm. If the protoplasm lacks any substance which is a normal constituent of it, a combination of outside material and membrane and consequent importation of substance within the cell will occur. The membrane will then function not only as a septum but also as the rest of the protoplasm of the cell. This brings us to another view of the osmotic behavior of cells in which a distinct Overton lipoid membrane is unnecessary. Protoplasm is a mixture of very complex colloidal substances suspended in a water medium. One of the properties of colloids as distinguished from crystalloids is their large molecular weight, and therefore large molecular volume. In protoplasm we probably have a combination of proteins, carbohydrates, and lipoids. The size of such a molecule would be enormous in comparison to the size of a crystalloid molecule. Due to the large size of the molecule we would not expect a high power of diffusibility and therefore homogeneity which is a criterion of a true solution. Protoplasm is then colloidal in nature and can not function as a true solution as Overton thinks. One of the most characteristic properties of colloids and one of the most important in a consideration of the physical properties of protoplasm is their property of lowering the surface tension of solutions with which they come in contact, and their consequent concentration at the surface. The concentration of such large molecules will result in the formation of a surface layer which approaches the solid state. These "pellicles", as Starling calls the surface layers, may be formed wherever there is a contact of two parts of the cell which are different in constituency (such condition can be easily imagined in a non-homogeneous liquid). The transference of substances, then, from the outside medium into the cell and from one part of the cell to another will be by osmosis thru these pellicles. According to this conception of membrane

formation (Starling) it is not necessary to think of a membrane which is of different composition from the rest of the protoplasm, as Overton, Höber, and Starling (Starling also thinks that this membrane becomes of different composition after its formation) think. It is just as simple to think of the whole protoplasm acting as a membrane due to the lipoid substances throughout it. This is the view that S. Loewe[20] holds. On the other hand, the hemolytic experiments carried on by Ransom show that the hemolysins act on the lecithin and cholesterol in the lipoid membrane. The analysis of the stromata of the red corpuscles, already referred to above, are given as another proof of the presence of a lipoid membrane. Such a membrane has never been detected, however, by any optical or staining means.

Besides the criticism of a distinct membrane, Overton's "Lipoid Theory" met with opposition on other grounds. According to this theory only basic dyes could enter the cell, for the acid dyes are not soluble in lipoids. Ruhland has found that some acid dyes can enter the cell. Overton's theory can be defended from criticism on this point on the grounds of the "mosaic structure" of the membrane. The main objection to Overton's theory is that he considers the lipoids as solution media in which the substance entering the cell dissolves. S. Loewe[21] carried on a series of experiments recently in which he attempted to prove the colloidal nature of lipoid substances and that due to their colloidal nature lipoids could not form solutions with the substances passing thru the membrane, but that they adsorbed them. The method he used was to dissolve the lipoid in an organic solvent, and the dye in water. He then stratified one solution over the other and studied the distribution of dye between water and lipoid. He used a similar method in the study of distribution of narcotics between lipoid and water. Loewe's results show

that the coefficient of distribution of dye and narcotic between lipoid and water, does not follow Henry's law of solution, but that of the adsorption isotherm. W. Koch,[22] Fischel, and A. P. Mathews,[23] on the other hand, explain lipoid function by chemical combination with the substance entering the cell.

Overton's "Lipoid Theory" is essentially a theory of narkosis, as it explains the entrance of narcotics into the cell very satisfactorily. The mode of action after the entrance of the narcotic, is, however, not so easily understood. Overton thinks that the narcotic changes the state of aggregation of the lipoid substances in the membrane, thereby changing the function of the latter. But he did not attempt to prove that the lipoids are the substances affected. W. Koch treated lecithin emulsions with various anesthetics and his conclusions are that there is no relation between the change in state of the lecithin particles and the rate of entrance of the narcotic into the cell. S. Loewe's observations are in agreement with Overton's theory in this respect. He measured the electrical resistance of lipoid membranes before and after introducing narcotics in the solutions which bathed the lipoid membranes. His results showed an increase of the resistance of the membrane to the current after introducing the narcotic. Loewe explains these results as due to the change of the lyophile to the lyophobe state of the lipoid particles in the membrane. In his theory of narkosis, then, he explains narcotic effect as due to the change in state of aggregation of the lipoid substances. With this change there is a consequent change in the function of the protoplasm and therefore a change in permeability, - the protoplasm losing its selective permeability and allowing substances to enter it that are harmful to its functions.

III. THE PURPOSE OF THIS INVESTIGATION

The theories of lipoid function in the process of adsorption and secretion and in narkosis are not by any means concordant, as we see from the above review. The reason for this can be found in the fact that these theories have not been founded on actual experiments with the lipoids themselves. A systematic study of the physical and chemical properties of the lipoids and their behavior under conditions that approach that of the living cell, has as yet not been attempted. It is the purpose of this investigation to make a beginning in this large field of study. The work connected with these experiments can be divided into two parts:

A. Preparation of Material

B. The Physical Properties of the Lipoids

1. Osmotic Pressure of Lipoid Solutions
2. Permeability of Lipoid Membranes
3. Nature of Combination between Lipoids and Other Substances
4. The Entrance of Dyestuffs into the Cell

IV. EXPERIMENTAL

A. Preparation of Material

Great care was taken in obtaining materials of a very high grade of purity. No particular method was followed in preparing any of the substances, but such lines were pursued by which the materials could be obtained in unoxidized form and free from other materials. Special care was taken to keep the substances that were easily oxidized from the influence of light and warm solvents and reagents that might in any way change their chemical composition.

Cholesterol. The macerated brain was spread on glass plates and dried in an air drier at a temperature of 37 degrees. The thoroughly dried brain was shaken with acetone for a day. The extract was pressed out and the residue again shaken with acetone. The process was repeated a third time. All the cholesterol was in that way extracted from the tissues. The purification of cholesterol was accomplished by crystallizing twice out of acetone and further by repeatedly crystallizing out of hot alcohol. The product obtained was a pure white substance of characteristic crystalline plates.

Kephalin and Lecithin. The residue from the cholesterol extraction was shaken for a day with benzene containing 5% absolute alcohol. The extract was pressed out and the whole process was repeated till the extract so obtained contained no kephalin or lecithin. Many other substances are extracted by the benzene and are mixed with the kephaline and lecithin.

The benzene was distilled off and the residue taken up in anhydrous ether in a tall cylinder. A white material settled to the bottom, which was "protagon," cerebrosides, sphingomyelin, a small amount of myelin, and sulphatids. The kephalin and lecithin remained in the clear ether solution, which was syphoned off. The residue was extracted with ether several times to remove all the kephalin and lecithin. The ether extracts were evaporated to a small volume in a vacuum dessicator (in the dark), and precipitated with acetone by very slowly pouring the ether solution into two and a half times its volume of acetone. The precipitate is in that way obtained in a flaky condition and can be dried more easily. By pouring the solution into acetone very slowly while stirring, the precipitate does not occlude so much impurity and can be purified by fewer precipitations. The acetone was quickly removed from the precipitate by decantation and filtered with suction. The precipitate was dried in a calcium chloride vacuum dessicator. The dried precipitate was again taken up in ether, freshly distilled over calcium chloride, and again allowed to settle, more of the white material separating out. The process of taking up in ether and separating the clear solution of lecithin and kephalin from the white material was repeated till the ether solution no longer contained any residue. In this operation thorough drying seems essential before taking up in ether, the protagon decomposition being accomplished in two or three operations if the precipitate is thoroughly dried before dissolving in ether. The kephalin and lecithin were in this way obtained free from the other substances present in the benzene extract. The separation of the two substances from each other was accomplished by precipitating the kephalin by means of absolute alcohol. The same precautions of precipitation and drying were taken here as in the case of the

acetone precipitations. The precipitation with alcohol was repeated three times, when the kephalin no longer contained any lecithin. A final precipitation of the kephalin was made with acetone as the latter is much more easily driven off than is alcohol. This prevents oxidation due to contact of the kephalin with alcohol for any considerable time. The kephalin obtained was a yellowish white, granular product.

The lecithin is left in the alcoholic filtrates from the kephalin. These are combined and the alcohol distilled off on the steam bath under reduced pressure. The residue is taken up in ether and the solution precipitated with acetone. The precipitate was dissolved in ether and the alcohol precipitation was repeated twice.

Erlandsen[24] and Thierfelder[25] extracted the dry brain with ether. This method has an advantage over the benzene method in that kephalin and lecithin can be obtained nearly free from sphingomyelin, cerebrosides, and sulphatids. On the other hand, kephalin and lecithin are more easily oxidized in an ether solution. These substances were therefore allowed to stand in ether as little as was possible. Thudicum extracts the brain with hot alcohol; the objection to this method is the use of a hot solvent on such unsaturated substances as kephalin and lecithin.

Sphingomyelin. The separation of the substances making up the white material from which kephalin and lecithin have been extracted, is a very difficult process, due to the fact that these substances affect each other's solubilities to a very marked degree. Moreover, very little is found in the literature on properties of these substances that can be made use of in their separation from each other. The method used for getting pure sphingomyelin followed Rosenheim's method to a small extent. The

greatest part of the separation was worked out in this laboratory, however, in such a way that the other substances found in the "white material", the cerebrosides and the sulphatids, could be obtained in pure form from the same material.

To obtain pure sphingomyelin the brain residue from which kephalin and lecithin and some white material were extracted by means of benzene containing 5% absolute alcohol, was treated several times with warm alcohol (60 degrees). The extract was each time pressed out, evaporated to a smaller volume, and allowed to crystallize out, at 0 degrees C. The substances that separated from the alcohol were sphingomyelin, sulphatids, cerebrosides, and protagon. They were filtered off and added to the white material that remained as a residue from the ether extract of kephalin and lecithin. These were again taken up in absolute alcohol and cooled to 0 degrees C. and filtered and extracted with ether several times to remove any lecithin or kephalin and other ether-soluble material. They were then dissolved in warm pyridine (45 degrees C.), allowed to cool, and filtered. The cerebrosides were left in solution and the treatment with pyridine was repeated three to four times. The bulk of the cerebrosides were thus removed. The loose combination of protagon was nearly completely broken up by the pyridine treatment. The precipitate of sphingomyelin and sulphatids was treated with a mixture of methyl alcohol and chloroform (1:3) at 45 degrees C. and allowed to cool to 0 degrees C. The sulphatid and some cerebrosides that were not dissolved by the pyridine crystallized out of the cold solution, while sphingomyelin remained in solution. The alcohol and the chloroform were distilled off and the sphingomyelin was dissolved in warm absolute ethyl alcohol and allowed to crystallize out at 0 degrees. This precipitate was then treated with a mixture of methyl alcohol and chloroform (4:1) and the sphingomyelin

was separated as before. The methyl alcohol-chloroform treatment was repeated till the sphingomyelin was obtained free from any cerebrosides or sulphatids.

The Cerebrosides

Cerebron. The pyridine which contained the cerebrosides in solution was distilled off and the residue as free as possible from pyridine was dissolved in a very small amount of absolute alcohol and allowed to crystallize out. A little acetone was always added to the alcohol filtrate to obtain the cerebrosides that had gone into the filtrate. The cerebrosides so obtained were again treated with pyridine and the process carried out as before. The cerebrosides obtained from the second pyridine treatment were then purified by the Thierfelder method. All such solvents as acetic acid, which Koch uses, were avoided, to guard against hydrolysis.

The Sulphatids

The sulphatids from which the cerebrosides were separated by pyridine and sphingomyelin by means of a chloroform and methyl alcohol mixture at 45 degrees, were again treated several times with chloroform and methyl alcohol and repeatedly treated with pyridine. The product obtained was a yellowish white powder when dried.

B. The Physical Properties of the Lipoids

1. The Osmotic Pressure of Lipoid Solutions

It is the property of colloidal solutions to give very small osmotic pressures. In fact, Starling and others, working on proteins came to the conclusions that colloids give no osmotic pressures when free from electrolytes. He found, however, that the serum proteins did give an osmotic pressure of about 30 mm. of mercury for 6% of proteins in the serum. Experiments were undertaken in this laboratory to study the osmotic pressures of lipoid solutions, first, with a view of proving the colloidal nature of the lipoid solutions, and, second, to study the relation of the lipoids to osmosis in the living cell. The cells used for these experiments were thistle tubes. The membranes were stretched tightly over the opening of the bell of the thistle tube. Great care was taken in making these membranes absolutely tight. The membranes were fastened by winding a linen thread very tightly around the flange of the tube, from one end of the flange to the other. A piece of cheese cloth was first tied over the opening, then the membrane to be used, and then another piece of cheese cloth. When cheese cloth was not used, the membranes were found to stretch under the weight of the solution inside of the cell. This was especially true in the case of rubber membranes. Rubber swells in organic solvents that it is permeable to, and so it is very essential to protect it from stretching in this swollen condition. The collodion membranes were made by stretching a piece of cheese cloth tightly over the opening of the bell, tying it to the thistle tube as described above and then dipping the cheese cloth into collodion several times, being careful not to get any of the collodion on the glass inside of the thistle tube, as it scales off the glass and makes the membrane faulty. Care was taken

to get a membrane which was as thin as possible and of uniform thickness. The lipoid membranes were precipitated on cheese cloth between two collodion membranes prepared as described above. The lipoid was in that way protected from being dissolved by the solvents used, thus making a longer time of experimentation possible and decreasing the error that the dissolved lipoid might introduce. The osmotic pressure of lecithin solutions in water and in organic solvents were measured against the pure solvents. The results are shown in numbers 1, 2, 3, 4, and 14, of Table I. The benzene solution rose to a height of 21 cm. and remained at that height for three weeks, to the end of the experiment. The pure water emulsions gave a very small rise - in fact, the rise was doubtful in the case of Number 1, where a parchment membrane was used. In Number 14, where a collodion membrane was used, the rise was higher and more certain. Water emulsions were then treated with narcotics, hypnotics, salt, acid, and alkali and their osmotic pressure compared with that of the pure water emulsion in Numbers 1 and 14. The results of these experiments are shown in Numbers 5, 6, 7, 8, 9, 10, 11, and 12, of Table I. These results show that narcotics and hypnotics affect very markedly the osmotic pressure of lecithin solutions. Moreover, in the two cases where alkali was used, the rise was in direct relation to the concentration. These results are not due to the hydrolysis of the lecithin by the alkali, as no hydrolysis products seemed to be present in the solution outside. Moreover, if the lipoid were gradually hydrolized into products that could pass thru the membrane, they would distribute themselves in such a way that their pressure on both sides of the membrane would be equal and so not affect the original height. Chloroform gives a negative osmotic pressure. The osmotic pressures given by kephaline solutions are similar to those given by lecithin, but they are not so high. The results are shown

No.	Solution Inside	Solution Outside	Membrane	Osmotic Rise	Time of Experiment
1	Water emulsion	Water	Parchment	.5 cm.	
2	3 gm. lecithin dissolved in 30 c.c. ether	Ether anhydrous	Rubber	7. cm.	7 days
3	3 g. lecithin dissolved in 30 c.c. petroleum ether	Petroleum ether	Rubber	6. cm.	8 days
4	3 gm. lecithin dissolved in 30 c.c. benzene	Benzene	Rubber	21. cm.	32 days
5	3% lecithin sol. in water + .25 gm. chloral hydrate in 150 c.c. solution	150 c.c. water + .25 gm. chloral hydrate	Parchment	6. cm.	14 days
6	3% lecithin containing 1 c.c. ether per 30 c.c. solution	Water containing 1 c.c. ether per 30 c.c.	Parchment	9.6 cm.	2 days
7	3% lecithin containing 1 c.c. chloroform per 30 c.c. solution	Water containing 1 c.c. chloroform per 30 c.c.	Parchment	-.5 cm.	
8	30 c.c. lecithin sol. in water containing 2 c.c N/2 NaOH	60 c.c. water containing 4 c.c. N/2 NaOH	Parchment	27. cm.	14 days
9	30 c.c. lecithin sol.containing 1 ccc. N/5 NaOH	Water containing 1 c.c. N/5 NaOH per 30 c.c.	Parchment	4. cm.	14 days
10	30 c.c. lecithin sol. in water + 2 c.c. N/2 H_2SO_4 inside	Water + 2 c.c. N/2 H_2SO_4 per 30 c.c. water	Parchment	0. cm.	14 days
11	30 c.c. lecithin sol. in water (3%) containing $\frac{1}{2}$ c.c. N/2 H_2SO_4	Water + $\frac{1}{2}$ c.c. N/2 H_2SO_4 per 30 c.c. water	Parchment	0. cm.	14 days
12	30 c.c. lecithin emulsion 4 c.c. N/10 NaBr	Water + 4 c.c. N/10 NaBr per 30 c.c. sol.	Parchment	0. cm.	14 days
14	30 c.c. lecithin emulsion	Water	Collodion	1. cm.	2 days

in Table II.

The results of the osmotic pressure experiments of sphingomyelin, cerebroson, and cholesterol, are given in Table III. Of these experiments only the sphingomyelin emulsion exerted an osmotic pressure. This may be due to the fact that the membranes used were permeable to the lipoid solutions. The chloroform solutions, however, give the same result as in kephaline and lecithin.

2. Permeability of Lipoid Membranes

In the consideration of the various osmotic theories, the semi-permeability of the membrane was intimated as the essential property of a membrane, upon which osmotic phenomena depend. A membrane is semi-permeable when it allows the solvent to go thru it but not the solute. According to Kahlenberg there is no truly semi-permeable membrane, because all membranes allow the passage of all substances thru them to a slight extent. Semi-permeability, according to him, is only a relative term. Be that as it may, what concerns us in osmotic phenomena, is the relative permeability of a membrane to substances, for it is that factor which determines which substances will pass out or into an osmotic or a living cell. What the main factor in semi-permeability of a membrane is is debated. Traube thought that the size of the pores of the membrane determines its semi-permeability. The evidence in favor of Traube's theory is the fact that large molecules like sugar, proteins, and other colloids, can not pass thru most membranes, while the smaller molecules can pass thru. The experiments of Reoult, Kahlenberg, Overton, and Tamman, show, however, that Traube's sieve theory can not, by itself at least, explain

TABLE II.

No.	Lipoid	Solution Inside	Solution Outside	Membrane	Viscosity	Osmotic Rise	Time
						cm.	
1	Kephaline	3% kephaline solution in water + chloral hydrate	Water and chloral hydrate	Collodion	5'35"	2.5	2 wks.
2	"	3% kephaline emulsion in water +2 c.c. N/2 NaOH	Water + NaOH in same conc. as inside	Collodion	5'15"	5	2 days
3	"	Kephaline emulsion in water .42 gm. urathane per 30 c.c.	Water + .42 gm. urathane per 30 c.c. water	Collodion		1.5	2 wks.
4	"	Kephaline solution containing .05 gm. chloral hydrate per 30c.c.	Water containing 1 c.c. chloralhydrate per 30c.c.	Collodion		2.5	2 "
5	"	30 c.c. kephaline emulsion in water +1 c.c. formaldehyde	Water +1 c.c. formaldehyde per 30 c.c. water	Collodion	4'59"	1	2 "
6	"	30 c.c. kephaline emulsion in water containing 1 c.c. ether	Water +1 c.c. ether per 30 c.c. water	Collodion		7	2 "
7	"	30 c.c. kephaline emulsion in water	Water	Collodion		1.5	2 "
8	"	3 gm. kephaline dissolved in 30 c.c. chloroform	Chloroform	Rubber	1'26"	Broke	2 "
9	"	3 gm. kephaline dissolved in 30 c.c. petroleum ether	Petroleum ether	Rubber	1'43"	28	1 2
10	"	3 gm. kephaline dissolved in 30 c.c. benzene	Benzene	Rubber	2'25"	35	2 "
11	"	3 gm. kephaline dissolved in 30 c.c. toluene	Toluene	Rubber	2'14"	32	2 "

TABLE III.

No.	Lipoid	Solution Inside	Solution Outside	Membrane	Osmotic Rise	Time
28	Sphingomyelin	Sphingomyelin in water	Water	Gold beaters skin	5.5	2 da.
29	Sphingomyelin	Chloroform solution of lecithin	Chloroform	Rubber	0	2 "
30	Cerebroside	Pyridine	Pyridine	Rubber	0	2 "
26	Cerebroside	.52 gm. cerebroside dissolved in chloroform	Chloroform	Rubber	0	3 "
25	Cerebroside	Cerebroside dissolved in water + a very small amount of lecithin	Water	Collodion	0	3 "
23	Cholesterol	Cholesterol dissolved in benzene	Benzene	Rubber	0	4 "
24	Cholesterol	Cholesterol dissolved in acetone	Acetone	Rubber	0	4 "
27		Cholesterol dissolved in chloroform	Chloroform	Collodion	0	4 "

the cause of semi-permeability. Raoult found that when he used pig's bladder to separate methyl alcohol and ether, the methyl alcohol passed thru the membrane into the ether. When he used a membrane of vulcanized caoutchouc, the flow was in the opposite direction. Tamman in this connection showed that rubber can take up ten times as much methyl alcohol as ether, while caoutchouc can take up one hundred times as much ether as methyl alcohol. Kahlenberg's experiments showed the same selective permeability of the membranes. Numerous other examples such as the passage of salt thru the cells of the intestine only in one direction can be cited to show that the sieve theory does not explain semi-permeability satisfactorily. The experiments of Kahlenberg, Tamman, Raoult, and Overton show clearly that the nature of the membrane and the substances in solution are factors in semi-permeability. Overton's experiments show the nature of the substances that a living cell is permeable or impermeable to, and from the solubility of various substances in fats, he draws the conclusion that the permeability of the cell membrane to a substance is related to the solubility of the substance in the membrane. He does not prove his theory, however, by direct experimentation with the lipoids themselves. The experiments below were undertaken with a view of studying the semi-permeability of lipoid membranes and the influence of other substances on their permeability. The method of making the lipoid membranes has already been described. The solution inside of the cell was albumen or cane sugar. These were chosen because it was supposed that a lecithin membrane would be impermeable to them. This was found to be true in both cases. For each lecithin-collodion membrane a control with the collodion membrane alone was run. Where the experiments were carried thru to the end, the cell with the lecithin membrane showed a higher rise than the cells with the collodion membrane.

Further, narcotics, salts and alkalis as a whole seemed to have the same effect when a lecithin membrane was used as when the lecithin emulsion was used inside of the cell with a parchment or collodion membrane. The results of the permeability experiments are shown in Tables IV and V.

3. The Nature of Combination Between Lipoids and Other Substances

As we have seen above, one of the chief points of Overton's theory is his postulation of a lipoid solution medium in which substances entering the cell must be soluble. We have also seen in the review of the properties of colloids that they do not form homogeneous solutions, which is a property Overton would give the lipoid substances. The osmotic experiments described above indicate the colloidal nature of these substances and therefore their inability to form true solutions. It was further attempted to prove the colloidal nature of lipoids by studying the distribution of dye between them and water. If lipoids form true solutions with the dye, Henry's law of solution, which says that $\frac{a - c}{C} = K$, ought to be obeyed. In this equation a is the original concentration of the dye in water, c, the end concentration of the dye in water, and K, a constant. The method used in these experiments is described below.

1. Kephaline Constant - Methylene Blue Variable

The kephaline was dissolved in petroleum ether (4 gms. in 500 c.c. petroleum ether), and 50 c.c. of this solution was placed in each of four Erlenmeyer flasks. A varied number of cubic centimeters of methylene blue made up to 100 c.c. with water was carefully poured down the side of the flasks so that no emulsion was formed. The flasks were then allowed to

TABLE IV.
Permeability of Lecithin Membranes

No.	Substances Added to Inside of Cell	Solutions Outside of Cell	Membrane	Rise
1	Water	N/10 NaBr	Collodion	4 cm.
2	Water	N/10 NaBr	Collodion & lecithin	---
3	1% albumen	Water	Collodion	---
4	1% albumen	Water	Collodion & lecithin	---
5	1% albumen + .05 gm. chloral hydrate per 30 c.c. solution	Water + .05 gm. chloral hydrate per 30c.c.	Collodion	?
6	Same as in No. 5	Same as No. 5	Collodion & lecithin	?
7	1% albumen + 1 c.c. chloroform per 30 c.c. solution	Water + 1 c.c. chloroform per 30 c.c.	Collodion	1.5 cm.
8	Same as No. 7	Same as No. 7	Collodion & lecithin	1. cm.
9	1% albumen + 1 c.c. anhydrous ether per 30 c.c. solution	Water + 1 c.c. anhydrous ether per 30c.c.	Collodion	---
10	Same as No. 9	Same as No. 9	Collodion & lecithin	---
11	1% albumen + 1 c.c. N/5 NaOH in 30 c.c. solution	Water +1 c.c. N/5 NaOH in 30 c.c.	Collodion	0
12	Same as No. 11	Same as No. 11	Collodion & lecithin	2 cm.
13	1% albumen + 1 c.c. N/10 H_2SO_4 per 30 c.c. solution	Water +1 c.c. N/10 H_2SO_4 per 30 c.c.	Collodion	0
14	Same as No. 13	Same as No. 13	Collodion & lecithin	2 cm.
15	Water	N/10 NaBr	Collodion	
16	Water	N/10 NaBr	Collodion & lecithin	- 1 cm.

TABLE V.
Permeability of Lecithin Membranes

No.	Solution Inside	Solution Outside	Membrane	Rise	Remarks
17	Water	50 c.c. $\frac{M}{5000}$ methylene blue made up to 250 c.c.	Collodion	0	No effect
18	Same as No. 17	Same as No. 17	Collodion-lecithin	0	All dye adsorbed
19	7% cane sugar sol.	Water outside	Collodion	1 cm.	
20	Same as No. 19	Same as No. 19	Collodion-lecithin	8 cm.	
21	7% cane sugar +1c.c. N/2 NaOH per 30 c.c. solution	Water +1 c.c. N/2 NaOH per 30 c.c.	Collodion	1 cm.	
22	Same as No. 21	Same as No. 21	Collodion-lecithin	4 cm.	
23	1% albumin	Water + 4c.c. N/10 NaBr to 240 c.c. water	Collodion	2.5	
24	Same as No. 23	Same as No. 23	Collodion-lecithin	4.5	
25	1% albumin + .05 gm. chloral hydrate per 30 c.c. solution	Water + .05 gm. chloral hydrate per 30 c.c. solution 4 c.c. N/10 NaBr	Collodion	1.5	
26	Same as No. 25	Same as No. 25	Collodion-lecithin	.5	
27	1% albumin 1 c.c.	Anhydrous ether	Collodion	7.5	
28	Same as No. 27	Same as No. 27	Collodion-lecithin	2	2 weeks

stand till equilibrium between the lipoid and water phase was reached. The concentration of the dye in the water phase was then determined by the colorimetric method. The results of this experiment are shown in Table VI.

TABLE VI.

Kephaline Constant - Methylene Blue Variable

Dye	Kephaline grams (M)	$\frac{a}{\text{in grams}}$	$\frac{c}{\text{in grams}}$	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene	.40	.03190	.00111964	25.672	.07695
Blue	.40	.01595	.0012409	11.853	.03677
per 100 c.c.	.40	.007978	.00067628	10.797	.01825
	.40	.004785	.00055506	7.6206	.01057

They bring out the important point that $\frac{a-c}{c}$ is not a constant, and that kephaline, therefore, has not the relation of a solution medium to methylene blue. Further, Curve 1 is not a linear curve and does not cross the axis at the zero point as a solution curve would. The curve does not go up vertically and then suddenly turn to right angles to the first direction, as the curve indicating chemical combination would. The curve clearly indicates adsorption between the lipoid and the dye.

2. Kephaline Constant - Methylene Violet Variable

The results of this experiment are similar to the one above. They are given in Table VII below.

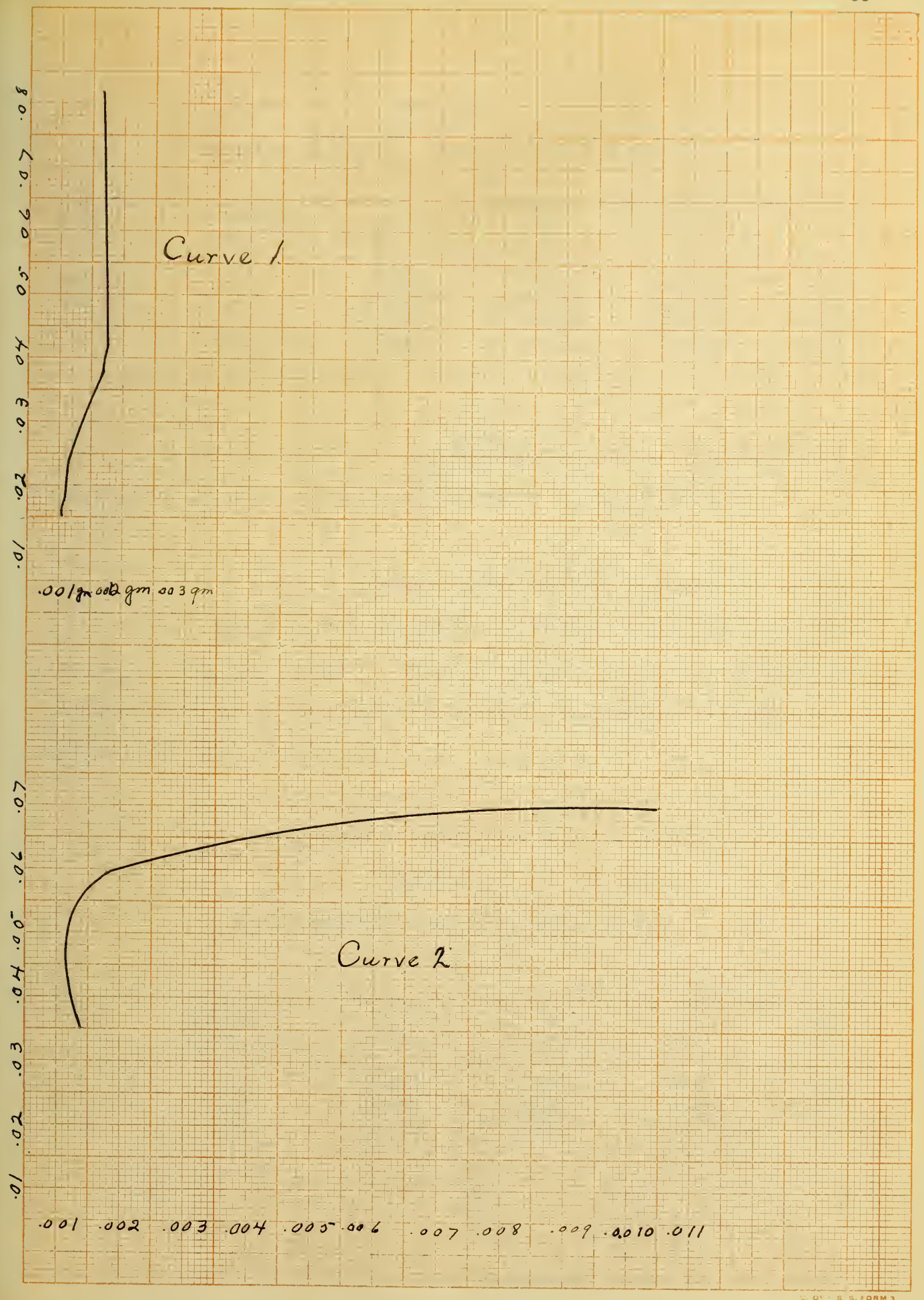


TABLE VII.

Kephaline Constant - Methylene Violet Variable

Dye	Kephaline grams (M)	a in grams	c in grams	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene	.40	.032025	.009992	2.2050	.05508
Violet	.40	.01920	.001310	13.6560	.04473
per 125 c.c.	.40	.01280	.0006597	18.4020	.03035
	.40	.008967	.0008903	9.0718	.02019

The values for $\frac{a-c}{c}$ are not constant and Curve 2 showing the relation for $\frac{x}{M}$ and c is an adsorption curve, as in Experiment 1.

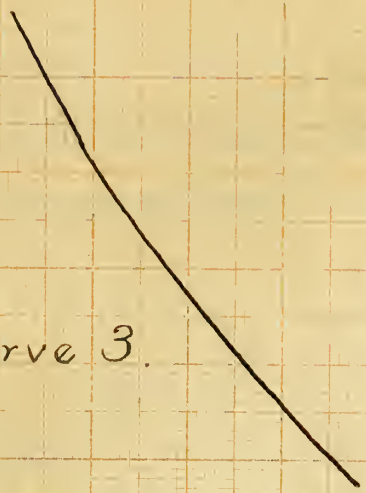
3. Lecithin Constant - Methylene Blue Variable

The results of this experiment are given in Table VIII. The values for $\frac{a-c}{c}$ are not constant. Curve 3 is not a solution or chemical combination curve, but a true adsorption curve. Its direction, however, is opposite from that of Curve 2.

TABLE VIII.

Lecithin Constant - Methylene Blue Variable

Dye	Lecithin grams (M)	a in grams	c in grams	$\frac{a-c}{c}$	$\frac{x}{M}$
	.40	.03190	.0051104	5.242	.06697
Methylene	.40	.01595	.0088044	0.81159	.01786
Blue	.40	.007978	.005869	0.35934	.0052725



Curve 3.

.001 .002 .003 .004 .005 .006 .007 .008 .009 .01

Curve 4.

.001 .002 .003 .004 .005 .006 .007 .008 .009

4. Lecithin Constant - Methylene Violet Variable

The values for $\frac{a - c}{c}$ of this experiment are not constant. Curve 4 is similar to Curve 2, and shows adsorption. The results are given in the table below.

TABLE IX.

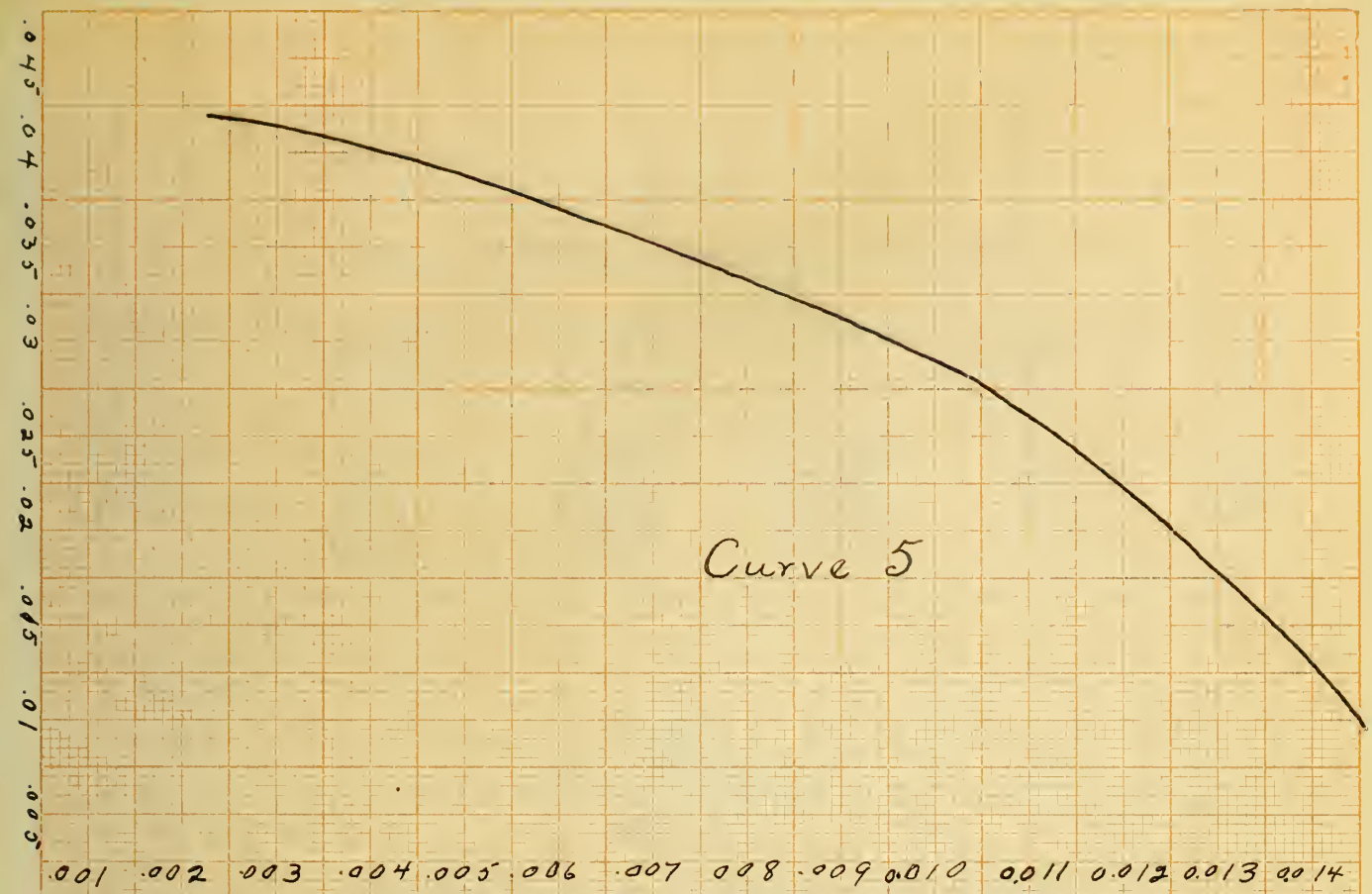
Lecithin Constant - Methylene Violet Variable

Dye	Lecithin grams (M)	$\frac{a}{}$ in grams	$\frac{c}{}$ in grams	$\frac{a - c}{c}$	$\frac{x}{M}$
	.40	.032025	.00637297	4.0197	.06411
Methylene	.40	.01920	.0062769	2.0597	.03231
Violet	.40	.01280	.0044194	1.8965	.02950
	.40	.008967	.0035243	1.5449	.01299

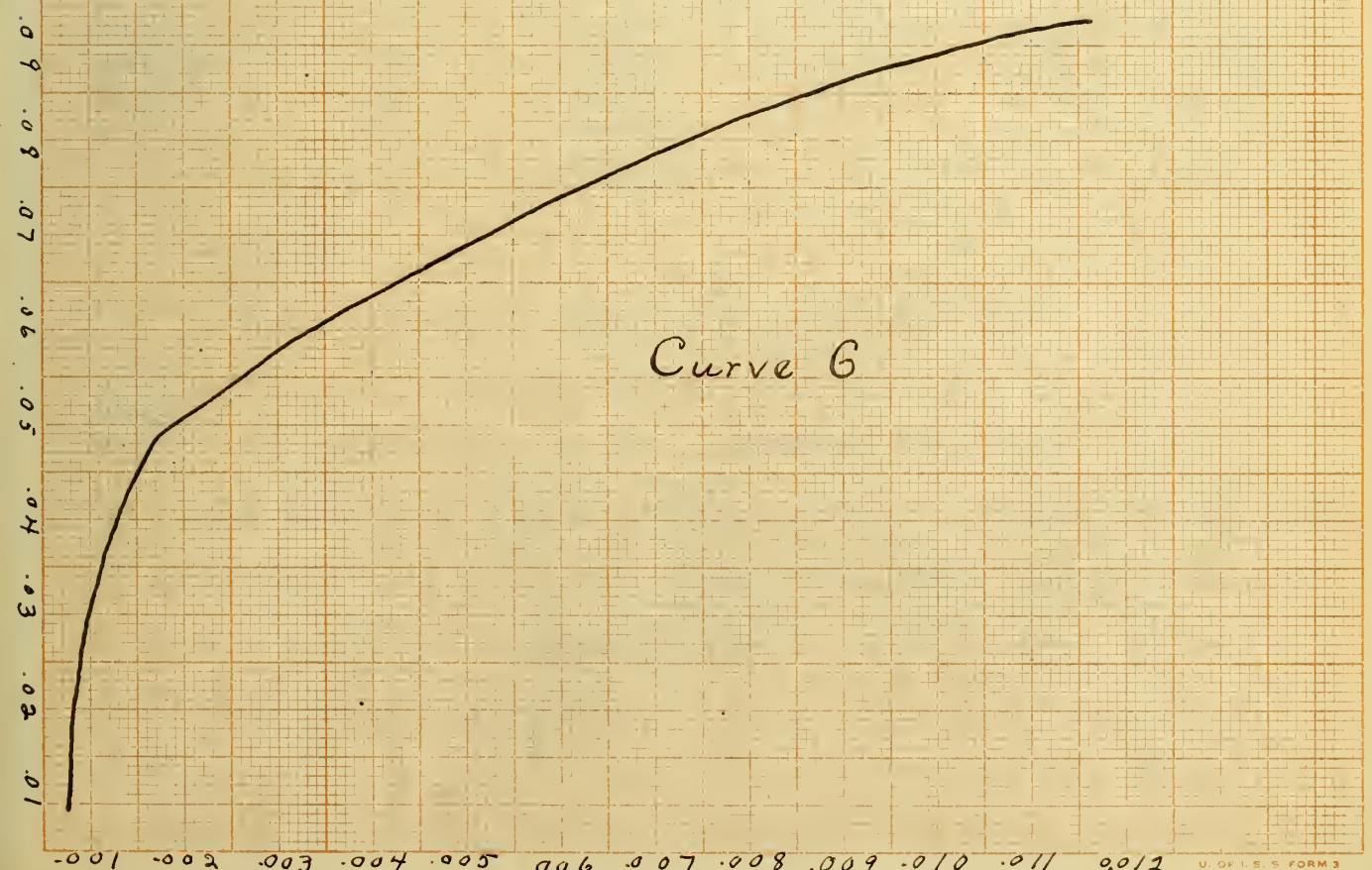
5. Kephaline Variable - Methylene Blue Constant

6. Kephaline Variable - Methylene Violet Constant

The results of these two experiments are given in Tables X and XI. The curves 5 and 6, for $\frac{x}{M}/c$, are very similar to the curves 3 and 4. The methylene blue in each case has an opposite slope from the methylene violet curves. This indicates that these curves are not only determined by the substance adsorbing but also by the substance adsorbed. The $\frac{a - c}{c}$ values show similar results to those of Experiments 1, 2, and 3.



Curve 5



Curve 6

TABLE X.

Kephaline Variable - Methylene Blue Constant

Dye	Kephaline grams (M)	$\frac{a}{\text{in grams}}$	$\frac{c}{\text{in grams}}$	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene	.375	.015950	.0017704	8.0273	.03781
Blue	.250	.015950	.009889	.6129	.02424
per 100 c.c.	.125	.015950	.0140998	.1322	.014801
	.075	.015950	.017609		

TABLE XI.

Kephaline Variable - Methylene Violet Constant

Dye	Kephaline grams (M)	$\frac{a}{\text{in grams}}$	$\frac{c}{\text{in grams}}$	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene	.375	.032025	.00021521	147.800	.08482
Violet	.250	.032025	.0011657	26.46	.12310
per 125 c.c.	.125	.032025	.0111048	1.9026	.16777
	.075	.032025			

7 and 8. Lecithin Variable - Methylene Blue

Methylene Violet Constants

Here again $\frac{a-c}{c}$ is not a constant and the Curves 7 and 8 are neither solution nor chemical-combination curves. Moreover, we find that the methylene blue and the methylene violet curves run in opposite directions, which is in agreement with the curves 3 and 4, and 5 and 6.. The results are found in Tables XII and XIII.

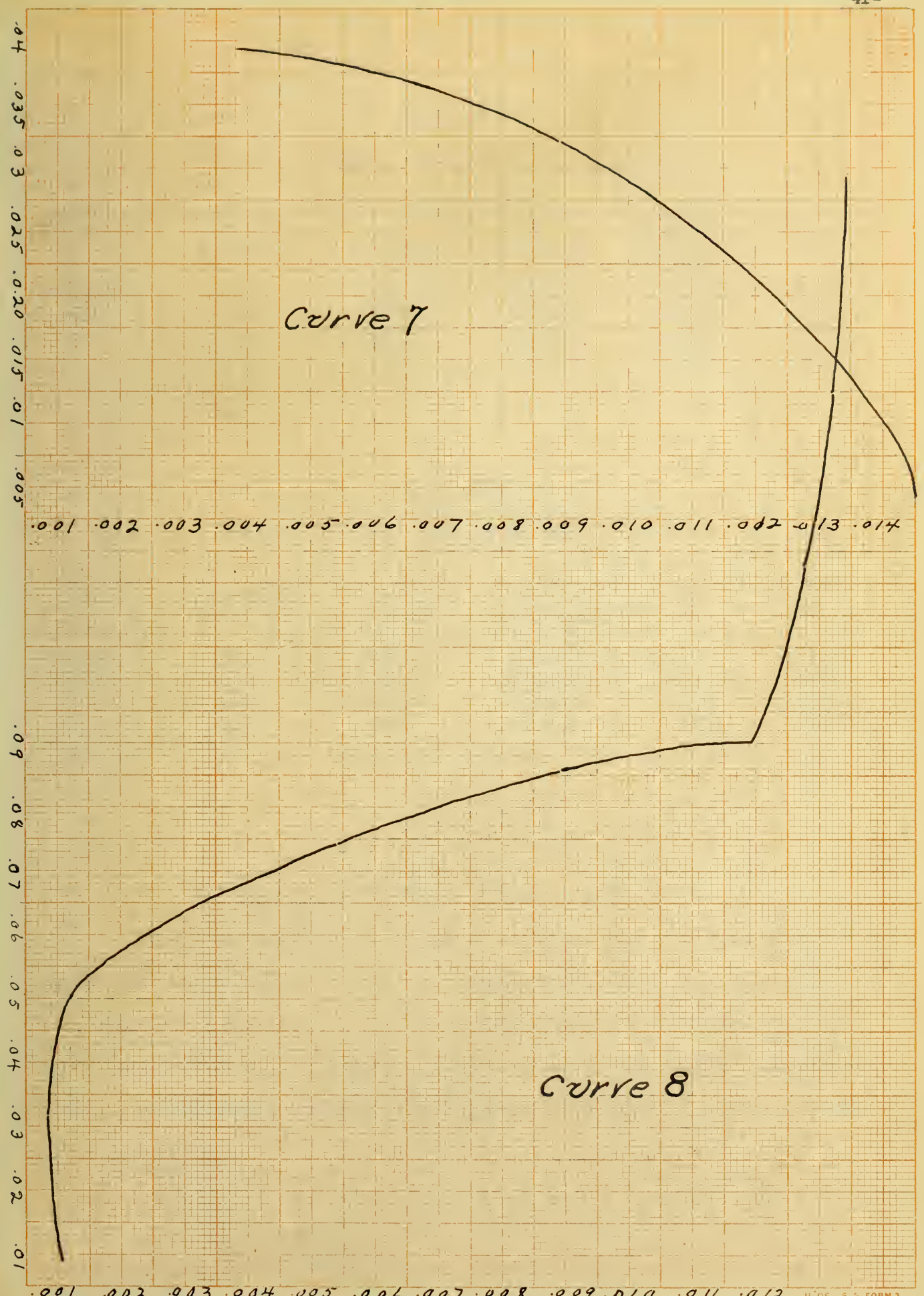

.04 .035 .03 .025 .020 .015 .01 .005
.001 .002 .003 .004 .005 .006 .007 .008 .009 .010 .011 .012 .013 .014
Curve 7
.09 .08 .07 .06 .05 .04 .03 .02 .01
.001 .002 .003 .004 .005 .006 .007 .008 .009 .010 .011 .012
Curve 8

TABLE XII.

Lecithin Variable - Methylene Blue Constant

Dye	Lecithin grams (M)	a in grams	c in grams	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene Blue	.375	.01595	.0033176	3.8075	.03368
	.250	.01595	.0079112	1.0111	.03215
	.125	.01595	.0128876	0.2382	.02450
	.075	.01595	.0139722	0.14155	.02637

TABLE XIII.

Lecithin Variable - Methylene Violet Constant

Dye	Lecithin grams (M)	a in grams	c in grams	$\frac{a-c}{c}$	$\frac{x}{M}$
Methylene Violet	.375	.032025	.00050599	62.30	.08405
	.250	.032025	.00066932	29.56	.12540
	.125	.032025	.0114009	1.8091	.16500
	.075	.032025	.0129381	1.4752	.25450

9. Cholesterol Constant - Methylene Blue Variable

This experiment was performed in a similar manner to the others, except that instead of letting the two phases stand quietly over each other till equilibrium was reached, the flasks were constantly shaken and readings taken from time to time to determine when equilibrium was reached. Cholesterol, however, showed no adsorption even at the end of two weeks. The results are given in Table XIV.

TABLE XIV.

Cholesterol Constant - Methylene Blue Variable

Dye	Cholesterol grams (M)	a in grams	c in grams
	.2	.01595	.01445
Methylene	.2	.009570	.009625
Blue	.2	.00638	.006390
	.2	.00319	.003250

10. Cerebron Constant - Methylene Blue Variable

The flasks in this experiment were shaken as in experiment 9. The solvent used was toluene instead of petroleum ether, as cerebron is insoluble in the latter solvent. Equilibrium was reached in five days. The results are given in Table XV.

TABLE XV.

Cerebron Constant - Methylene Blue Variable

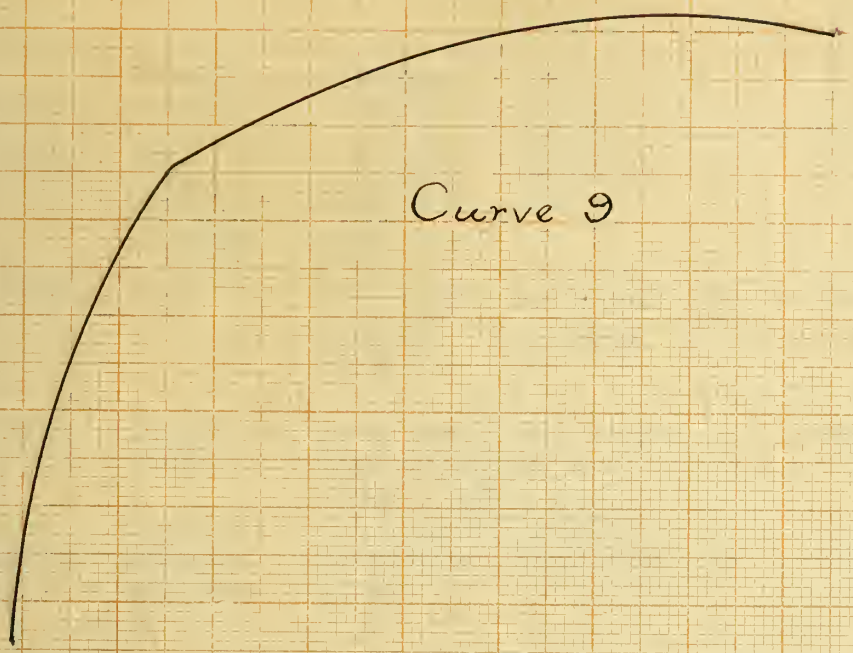
Dye	Cerebron grams (M)	a in grams	c in grams	$\frac{a-c}{c}$	$\frac{x}{M}$
	.8	.01595	.010377	.5370	.006966
Methylene	.8	.007975	.003634	.666	.00542
Blue	.8	.00319	.001917	.664	.00159

No permanent emulsions were formed with the organic solvent and water in either experiment 9 or 10.

Although the values for $\frac{a - c}{c}$ approached a constant, Curve IX representing $\frac{x}{M}/c$ is not a solution curve, since it is not linear and does not cross the zero point. Moreover, it does not indicate a chemical combination but is of the form of a true adsorption curve.

4. The Permeability of Membranes to Dyestuffs

The study of the permeability of membranes to dyestuffs was undertaken because it was considered a good method of studying the general permeability properties of membranes, as well as because the entrance of dyes into cells are of importance from a toxicity standpoint. In these experiments different membranes were used, and also different solutions inside the cell. In all the cells where lipoid membranes were not used, there was a diffusion of the dye thru the membrane and the solutions inside became colored. In the cells that had lipoid membranes (2, 5, 6) the methylene blue was adsorbed by the membrane, leaving the water outside colorless. The solution inside the cell in all three cases remained colorless. An osmotic pressure was shown in only one of these experiments (6), where the lipoid solution was used inside the cell with a lipoid membrane. Evidently that membrane fulfilled the qualifications of semi-permeability more perfectly. This experiment, however, agrees with Ruhland's[26] that the entrance of dyes into a cell can not be thru a lipoid membrane, because such a membrane would hold the dye. The results are shown in Table XVI.



Curve 9

.001 .002 .003 .004 .005 .006 .007 .008 .009 .010 .011

.001 .002 .003 .004 .005 .006 .007 .008 .009 .010

TABLE XVI.

Permeability of Membranes to Dyestuffs

No.	Lipoid	Solution Inside	Solution Outside	Membrane	Rise	Time	Remarks
1	Lecithin	Lecithin emulsion in water	Methylene blue in water	Parchment	0	5 da.	Diffusion of dye thru membrane
2	Lecithin	Water	Methylene blue in water	Collodion-lecithin	0	5 da.	All dye adsorbed by membrane
3	Kephalin	Kephalin emulsion	Methylene blue in water	Parchment	0	5 da.	Diffusion of dye thru membrane
4	Kephalin	Kephalin emulsion	Methylene violet	Parchment	0	5 da.	Diffusion of dye thru membrane
5	Kephalin	Water	Methylene blue in water	Gold beater's skin	0	5 da.	Diffusion of dye thru membrane
6	Kephalin	Water	Methylene blue in water	Gold beater's skin - kephalin	0	5 da.	Adsorption of dye by membrane
7	Kephalin	Kephalin emulsion in water	Methylene blue in water	Gold beater's skin - kephalin	11 cm.	5 da.	

V. DISCUSSION OF RESULTS

The results on osmotic pressure of lipoids show that lipoid solutions are colloidal since their osmotic pressures are very small in comparison to the same concentrations of crystalloid solutions. On the other hand, these experiments agree with Moore and Roaf that colloids give osmotic pressures that are not due to crystalloids as impurities, since the substances used for these experiments were highly purified. Moreover, as Moore and Roaf say, any rise due to crystalloids would not be constant, since the crystalloids would diffuse thru the membrane till the osmotic pressures they exerted would be equal on both sides of the membrane.

Secondly, these results show that hypnotics, narcotics, and alkalis change the state of the lipoid particles in a water emulsion, since they caused a rise in osmotic pressure. What the nature of that change is can not be judged from these experiments. The viscosity determination on a few of these solutions so treated, however, show a reduction in molecular friction, since the viscosity was lowered in every case. This would seem to indicate a dissociation of the lipoid particle into smaller particles. The effect on treating the water emulsion with the substances mentioned above was similar to the effect of dissolving the lipoids in those substances directly, except that the effect of the latter was greater.

The results on the study of permeability and the effects of salts, acids, bases, narcotics, and hypnotics, on permeability and consequent change in the osmotic pressure, agree with Overton's and Loewe's theory that the state of aggregation of the lipoid membrane or solution is changed. These

results show clearly that the lipoids function in the process of osmosis in the living cell. The lipoids formed semi-permeable membranes, and their degree of permeability is changed by different substances. Of the substances used only chloroform had a negative influence on all the lipoids. This may be due to the fact that the chloroform combined in some way with the lipoid and formed a larger molecule. The result seems to be similar to the adsorption of the dye by the lipoid membrane without any rise of the liquid in the cell.

The experiments on the study of the entrance of dyestuffs into a cell show, first, that lipoid membranes adsorb the dye and therefore do not allow them to enter the cell; secondly, they show that a colloidal solution inside the cell is necessary for the production of an osmotic pressure, for in number 3, where the lecithin-gold beater's skin membrane and a lecithin solution inside was used, an osmotic pressure was produced, while in 1 and 2 there was no osmotic effect. Whether the kephaline (lipoid) emulsion was the essential, or whether any substance to which the lipoid membrane is impermeable would have produced the same effect is still to be proven.

The results of experiment 3 agree with Loewe that the lipoid substances do not form true solutions with dyes, but that the process is one of adsorption. This is another indication of the colloidal nature of the lipoid bodies. Whether this is true in the case of all substances passing thru a lipoid membrane, however, is still to be proven. It must not be forgotten that dyes are themselves colloidal in nature, and that, therefore, it seems possible that the nature of combination between lipoid and crystalloid substances, for instance, is different from that proven for dyes. Moreover, in the experiment on the permeability of lipoid membranes to dyes, it has been shown that the membrane adsorbed the dye and that the latter in no case

entered the cell, even when the dye outside was in excess of the amount the membrane could adsorb. It would seem, then, that lipoid membranes formed a lipoid-dye combination which was impermeable to the dye itself. It would not seem, then, that all substances entering a cell are adsorbed by the lipoid bodies, for they would then never reach the cell sap. On the other hand, a solution of the lipoid bodies with the substances entering the cell, as Overton thinks, can not take place, as the lipoid solutions seem to behave as colloidal solutions, unless their physical nature is changed under the influence of other substances. The effect of narcotics and hypnotics on permeability of membranes and on the rise in the osmotic pressure seems to indicate that this may be true.

The results of the adsorption of dyes by cholesterol do not agree with Loewe's results, as the latter show an adsorption of the dye. This may be due to some impurity in the cholesterol that Loewe used.

VI. SUMMARY

1. Solutions formed by lipoids are colloidal.

2. Lipoids' solutions produce an osmotic pressure.

3. Narcotics, hypnotics, and alkalis change the physical properties of lipoid emulsions, as shown by change in their osmotic pressures and their viscosity.

4. Narcotics, hypnotics, and alkalis change the permeability of lipoid membranes.

5. Lipoids combine with methylene blue and methylene violet by adsorption and not chemically or as a solution.

6. Dyes can not enter a cell with a lipoid membrane, because the latter adsorb them and form a membrane impermeable to the dye.

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